of rejection. For clarity, applicants have herein amended each recitation of "binding partner polypeptide" to "second, binding partner polypeptide."

Claim 1 is said to be indefinite for recitation in subsection (d) of the phrase "contacting said immobilized polypeptide and said binding partner polypeptide with said sample." The Office Action asks "how do the second polypeptide recited in subsection (c) differ from the binding partner polypeptide of subsection (d)?" Applicants submit that the amendment of claim 1, subsection (a) to refer to "a second binding partner polypeptide" is sufficient to clarify to what subsection (d) is referring.

Claim 1 is said to be indefinite for recitation of the phrase, in subsection (e), "assaying the modification of at least one of the polypeptides." The Office Action asks "What causes the modification of one of the polypeptides?" Applicants submit that, as stated in the preamble, the claim is drawn to "a method for detecting, in a sample, the presence of a modifying enzyme which covalently modifies a polypeptide." Thus, when such an enzyme is present in a sample, which is what the claimed method seeks to determine, the "modifying enzyme which covalently modifies a polypeptide" causes the covalent modification of one of the polypeptides. When the sample lacks such an enzyme, the assay detects no covalent modification of one or both of the polypeptides, thereby indicating the absence of such a modifying enzyme. Applicants respectfully request reconsideration and withdrawal of this rejection under §112, second paragraph.

In view of the above, applicants submit that claim 1 is definite and respectfully request the reconsideration and withdrawal of the rejections of this claim under §112, second paragraph.

Claim 13 is rejected as indefinite for reciting contacting one or both of said first polypeptide and said binding partner polypeptide with an agent capable of modifying one or both of said polypeptides. Applicants submit that the cancellation of this claim herein renders this rejection moot.

Claim 15 is rejected as indefinite for use of the phrase "measured in real time." The Office Action asks "What amount of time the assay is measured in is not distinctly claimed.

How does real time differ from time?" Applicants submit that the specification defines "real time" as follows:

> As used herein in reference to monitoring, measurements or observations in assays of the invention, the term "real-time" refers to that which is performed contemporaneously with the monitored, measured or observed events and which yields a result of the monitoring, measurement or observation to one who performs it simultaneously, or effectively so, with the occurrence of a monitored, measured or observed event. (Page 16, lines 17-21)

In view of this express definition of the term "real time," applicants submit that it is clear that the claim requires that the results of the measurement of the association of the polypeptides are provided simultaneously, or effectively simultaneously, with the occurrence of that association. Applicants respectfully request the reconsideration and withdrawal of this rejection under §112, second paragraph.

Claim 17 is rejected as indefinite because the term "said binding partner polypeptide" lacks antecedent basis. Applicants submit that the amendment of claim 17 to recite "A polypeptide pair comprising a first polypeptide immobilized to a support, ands a second *binding partner* polypeptide bound to the first polypeptide" (emphasis added) is sufficient to overcome this basis of rejection. For consistency, the reference to "said binding partner polypeptide" is also amended to recite "said second binding partner polypeptide."

Claim 17 is rejected as indefinite for recitation of the phrase "is required for association." The Office Action states that "the phrase refers back to both the phrase 'covalent modification' and 'results in modulation,'" and that "which of these two phrases 'is required for said association' refers is unclear." Applicants submit that as amended, the entire clause in question reads as follows:

> "covalent modification of at least one of the polypeptides results in modulation of the binding, and is required for said association of said first polypeptide and said second binding partner polypeptide."

Applicants submit that, contrary to the assertion of the Office Action, the phrase "is required for said association" does not refer back to the phrase "results in modulation." Rather, the phrase "is required for said association" refers back only to "covalent modification." As written, the "covalent modification" both "results in modulation of the binding, *and* is required for said

association" (emphasis added). The inclusion of the emphasized word "and" clearly makes the phrase "is required for said association" refer back to the "covalent modification" and not to "results in modulation." Applicants respectfully request reconsideration and withdrawal of this rejection under §112, second paragraph.

Claim 17 is rejected as indefinite for recitation of the phrase "the binding of the polypeptides is detectable." The Office Action asks "What does the binding if the polypeptides are already bound to one another? What binds to the polypeptides and is detectable? What is detectable, the polypeptide pair or what binds to the polypeptide pair? How can a pair of polypeptides comprise a third polypeptide that is detectable and be considered a pair?"

Applicants submit that the claim is drawn to a polypeptide pair "comprising a first polypeptide immobilized to a support, and a second binding partner polypeptide bound to the first polypeptide," and that a usefulness of the claimed polypeptide pair bound to each other is for monitoring the activity of a modifying factor, e.g., an enzyme. As claimed, the covalent modification of at least one of the polypeptides by the modifying factor "results in modulation of the binding" of the polypeptide pair. In order to be useful in this manner, the binding of the polypeptides must be detectable, as recited in the claim. That is, a modulation of the binding of the claimed pair of polypeptides to each other reports on the activity of a modifying factor being monitored with the claimed pair.

Applicants submit that the preamble already requires that the claimed polypeptide pair is bound *to each other*, and that it is thus clear that this is the binding that is recited to be detectable. The fact that the polypeptides are bound to each other is also acknowledged in the Office Action with the question "What does the binding if the polypeptides are already bound to one another?" Thus, Applicants believe that it is clear that it is the binding of the polypeptides *to each other* that is detectable – that is, there is no third polypeptide. In order to avoid any question, however, Applicants have amended the claim to recite "wherein the binding of the polypeptides *to each other* is detectable."

In view of the above, Applicants respectfully submit that claim 17 as amended is definite, and respectfully request the reconsideration and withdrawal of the rejections of this claim under §112, second paragraph.

Claim 21 is rejected as indefinite because the claim "provides first and second polypeptides and a test sample, but they are not combined." Applicants have amended claim 21 herein to recite "providing said second polypeptide and said test sample, and combining said second polypeptide and contacting said test sample with said first polypeptide immobilized on a support." Applicants submit that the amendment is sufficient to overcome this §112, second paragraph rejection, and respectfully request reconsideration of the claim.

Rejection of claims under 35 U.S.C. §102

Rejection of claim 17 under 35 U.S.C. §102(e), over Mills et al.:

Claim 17 is rejected under 35 U.S.C. §102(e) as being anticipated by Mills et al. (U.S. 5,773,592). The Office Action states "It is the position of the Examiner that Mills et al. discloses a composition of 'a polypeptide pair comprising a first polypeptide immobilized to a support and a second polypeptide bound to the first polypeptide', wherein the first and second polypeptides of Mills are bound to each other through a biocompatible polymer." The Office Action concludes that Mills et al. discloses an immobilized polypeptide pair, wherein covalent modification of one of the polypeptides results in modulation of association, and results in a detectable signal, and that this anticipates the claimed invention. Applicants respectfully disagree.

Applicants submit that the Mills et al. reference does not teach a first polypeptide immobilized to a support and a second polypeptide binding partner polypeptide bound to the first polypeptide, as required by the claim. Specifically, the reference does not teach the first polypeptide *bound to* the second polypeptide. Applicants submit that Mills teaches a polypeptide pair comprising insulin tethered to glucose oxidase via a biocompatible polymer. The binding of glucose to glucose oxidase modulates the release of insulin. Mills also teaches a polypeptide pair comprising tissue plasminogen activator tethered to xanthine oxidase via a biocompatible polymer. The binding of xanthine to xanthine oxidase modulates the release of tissue plasminogen activator. Applicants submit that the tethering of one polypeptide to another "through a biocompatible polymer" taught by Mills et al. is not "a second polypeptide bound to the first polypeptide" as required by claim 17. Rather, the arrangement taught by Mills et al. is a first polypeptide, e.g., insulin, bound to a biocompatible polymer, and the second polypeptide,

e.g., glucose oxidase, bound to the biocompatible polymer. Applicants submit that the instant specification defines "binds" or "associates" as follows:

> As used herein, the term "associates" or "binds" refers to polypeptides as described herein having a *binding constant* sufficiently strong to allow detection of binding by a detection means, such as FRET or surface plasmon resonance. Preferably, the polypeptides, when associated or bound, are in *physical contact with each other* and have a *dissociation constant* (Kd) of about 10μM or lower. The ***contact region*** *may include all or parts of the two molecules*. (See specification page 10, lines 20-25; emphasis added)

The specification thus requires that there be a "contact region" between the first and second polypeptides, that contact region including "all or parts of the two polypeptides" – i.e., the polypeptides must be bound *to each other*. Applicants submit that the biocompatible polymer-tethered polypeptides taught by Mills et al. are not bound to each other by a contact region. Thus, they are not "bound" to each other as that term is understood in light of the specification. As such, Mills et al. does not teach all elements of claim 17 and cannot therefore anticipate the claim. Applicants respectfully request that the §102(e) rejection of claim 17 over Mills et al. be reconsidered and withdrawn.

Rejection of claims 1, 2, 14, 16, 18 and 20 under 35 U.S.C. §102(e), over Avruch et al.:

The Office Action states that the Avruch et al. reference (U.S. patent No. 5,582,995) "discloses a polypeptide pair wherein covalent modification of at least one of the polypeptides results in modulation of the association of the polypeptides of the pair, wherein a polypeptide pair is disclosed, one being a transferase which covalently modifies a polypeptide substrate that is immobilized, and covalent modification of the immobilized substrate results in modulation of the transferase enzyme/polypeptide substrate association, specifically dissociation from the substrate. The covalent modification of the polypeptide results in an association that is specific between the polypeptide and the enzyme and a farnesyl residue, wherein the covalent addition of a farnesyl residue modulates the association of the enzyme and the farnesyl residue containing substrate (see col. 7, lines 7-27 and col. 8, lines 28-29)." The Office Action thus concludes that the Avruch et al. reference anticipates the claimed invention. Applicants respectfully disagree.

First, Applicants submit that the passages cited at col. 7, lines 7-27 and col. 8, lines 28-29 of the Avruch et al. reference do not teach measurement of the association of a farnesyl

transferase polypeptide with an immobilized substrate. While the reference teaches that Ras can be farnesylated at residue C_{186}, the reference does not teach measuring the association of the farnesyl transferase to the Ras polypeptide, which forms the basis of the Office Action's conclusion of anticipation. Rather, the reference teaches assays designed to measure the association of Ras and Raf. In vitro binding experiments involved incubation of wild-type or mutant GST-Raf, immobilized on glutathione agarose beads, with GTPγS-loaded recombinant V12GrasH or GDPβS-loaded V12GrasH. After binding, the washed beads were analyzed for binding of Ras polypeptide by SDS-PAGE and immunoblotting with a Ras monoclonal antibody (col. 7, lines 7-27, cited by the Office Action). The cited text at col. 8, lines 28-29 relates only to the monoclonal pan-Ras antibody used for immunoblotting.

The passage cited at column 6, lines 1-47 describes additional Ras-Raf binding assays using Raf GST fusions and recombinant Ras polypeptides. Applicants submit that none of these assays describes a method for detecting, in a sample, the presence of a modifying enzyme which covalently modifies a polypeptide. The reference merely describes basic protein:protein association assays that examine the effect of various Raf mutations on the association. Further, The association of GTP and GDP with the Ras polypeptide is not covalent, so this cannot constitute a covalent modification as required by the claim.

The passage cited at column 8, lines 50-54 examines the ability of recombinant wild-type and mutant Raf polypeptides to alter Ras GTPase activity in the presence and absence of Ras-GAP. The GTPase activity is estimated from the amount of labeled GTP remaining bound to Ras. This does not teach a pair of polypeptides capable of associating, wherein the covalent modification of at least one of the polypeptides results in modulation of the association and is required for the association of the first polypeptide and the second binding partner polypeptide. There is no covalent modification described, other than possibly the hydrolysis of GTP to GDP, which is not covalent modification of a polypeptide.

Finally, the description of screening assays based upon the ability of Ras to bind Raf (cited at col. 13, lines 25-67 and col. 14, lines 1-36) do not anticipate the claimed invention. The passages cited first describe two hybrid screening assays and screening assays examining the binding of an agent to a single polypeptide. These obviously do not satisfy the requirements of

the claims (col. 13. lines 25-55). The passages then describe the use of Ras and Raf polypeptides to screen for compounds that disrupt the binding of Ras to Raf. Applicants submit that these assays do not detect the presence of a modifying enzyme which covalently modifies a polypeptide, as recited in the claim. Further, they do not teach a pair of polypeptides wherein the covalent modification of at least one of the polypeptides results in modulation of the association and is required for the association of the first polypeptide with the second binding partner polypeptide, as required by claim 1 as amended.

Similarly, Avruch et al. does not teach the situation wherein the covalent modification of one or both polypeptides in a binding pair, by a modifying agent, results in modulation of the binding of the pair of polypeptides to each other, as required by claim 18. As such, Avruch et al. does not anticipate claim 18 or claim 20 which depends from it.

In view of the above, Applicants submit that Avruch et al. does not anticipate claims 1 as amended, 2, 14, 16, 18 or 20. Applicants respectfully request the reconsideration and withdrawal of the rejection of these claims under §102(b) over Avruch et al.

Rejection of claims 1, 2, 10, 11 and 20 under 35 U.S.C. §102(e), over Josiah et al.:

Claims 1, 2, 10, 11 and 20 are rejected under §102(e) over Josiah et al., U.S. patent No. 6,146,842. The Examiner has previously stated that "Josiah disclose a method of assaying a sample that utilizes the following components. The two polypeptides are a protein substrate, (see claim 12) and farnesyl or geranylgeranyl transferase. (see claim 13) The protein substrate is immobilized prior to contact with the sample (claim 19) and is susceptible to covalent modification (see claim 14). The sample of Josiah is a test compound. (claim 12)."

The present Office Action maintains the prior rejection over Josiah et al., stating: "It is the position of the Examiner that upon binding of the enzyme to the substrate and the covalent addition of either farnesyl or prenyl group to the substrate, the binding of the enzyme to the substrate is modulated such that the enzyme will dissociate from the modified substrate. The covalently modified substrate would evidence the presence of a detectable label due to the covalent addition of the farnesyl or prenyl group to immobilized first polypeptide. The detectable label permits the assaying of the modification of the immobilized substrate due to the association of the binding partner (enzyme) and the immobilized first polypeptide substrate. The

association is required for the covalent modification of the immobilized first polypeptide and is also required for the addition of a detectable label to the immobilized substrate so that the covalent modification can be assayed through the presence of the label associated with the covalently added group." Applicants respectfully disagree.

First, claim 1 as amended recites "measuring the association of the second binding partner polypeptide to the first polypeptide, thereby determining the modification of at least one of the polypeptides, whereby the presence of a modifying enzyme is detected." Applicants submit that Josiah et al. does not measure the association of the second binding partner polypeptide to the first polypeptide, thereby determining the modification of at least one of the polypeptides, but rather measures the covalent modification directly. Applicants submit that these are two different things. Where one directly measures the modification of one of the polypeptides, as done by Josiah et al., there is no need to measure the association, as the presence of the covalent modification demonstrates the presence of a modifying enzyme. However, the claimed approach based upon measurement of the association evidences the presence of an enzyme by its effect on the association of the pair of polypeptides, not by measurement of the covalent modification itself.

The difference between the measurement of the covalent modification itself (e.g., the measurement of the addition of a farnesyl residue by detecting labeled farnesyl residue) and detection of a change in association of two polypeptides as a result of such covalent modification is apparent in the statements of the Office Action. The Office Action states that "the association is required for the covalent modification of the immobilized first polypeptide and is also required for the addition of a detectable label to the immobilized substrate." This is not what is required by claim 1. Rather than requiring that the association is required for the covalent modification of the immobilized first polypeptide, the claim requires that "the covalent modification of at least one of the polypeptides results in modulation of the association *and is required for the association of the first polypeptide and the second binding partner polypeptide.*" That is, the claim demands that *covalent modification is required for the association*, not that association is required for modification. This is the exact opposite of what the Office Action extracts from the Josiah reference. The claim makes it clear that *there is no association without the covalent modification* of at least one of the polypeptides. Thus, the association of the farnesyl transferase

enzyme of Josiah with the immobilized substrate when the enzyme farnesylates the substrate does not fall within the requirements of the claim, because the enzyme will not associate with the substrate if the substrate is already farnesylated. As such, Applicants submit that the teachings of Josiah et al. do not satisfy the requirements of claim 1 as amended, and therefore cannot anticipate claim 1 or claims 2, 10, 11 and 20 that depend from it.

In view of the above, Applicants submit that claims 1, 2, 10, 11 and 20 are not anticipated by Josiah et al., and therefore respectfully request the reconsideration and withdrawal of the rejection of these claims under §102(e) over the Josiah et al. reference.

Rejection of claims 1-3, 5, 10, 12-17 and 20 under 35 U.S.C. §102(e), over Shone et al.:

Claims 1-3, 5, 10, 12-17 and 20 are rejected under 35 U.S.C. §102(e) as being anticipated by Shone et al. (U.S. 5,962,637). The Office Action states that Shone et al. disclose a method of detecting the presence of a modifying enzyme in a sample, wherein the enzyme modifies a polypeptide covalently, and the enzyme is botulism or tetanus toxin. According to the Office Action, the first polypeptide required by, for example, claim 1, is a substrate polypeptide and the binding partner polypeptide is an antibody capable of binding the substrate after covalent modification with an enzyme. The Office Action states that the claimed step of contacting the immobilized polypeptide with the second polypeptide is met by Shone's teaching of contacting an immobilized polypeptide with the test compound. Applicants respectfully disagree.

Applicants submit that Shone et al. does not teach a method wherein the covalent modification of at least one of the polypeptides comprises one of the group consisting of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications, as required by claim 1 as amended. The language of the amendment is supported at page 4, lines 6-10 of the specification. Applicants submit that the Shone et al. reference teaches an assay for the botulinum toxin and tetanus toxin proteases, using an antibody that only binds cleaved protease substrate. Protease cleavage is not encompassed by claim 1 as amended. Thus, the Shone et al. reference cannot anticipate claim 1 or its dependents 2, 3, 5, 10, 12-16 and 20.

Regarding the rejection of claim 17 over the Shone et al. reference, Applicants submit that the reference does not teach the necessary elements of the claim as amended herein. Specifically, the Shone et al. reference teaches proteolytic cleavage of the immobilized substrate, whereas claim 17 as amended does not include proteolytic cleavage as a covalent modification permitted by the claim. As such, the Shone et al. reference cannot anticipate claim 17 as amended.

In view of the above, applicants submit that claims 1-3, 5, 10, 12-17 and 20 are novel over the Shone et al. reference. Applicants respectfully request reconsideration and withdrawal of the rejection of these claims under §102(e) over Shone et al.

Rejection of claims 1-7, 12-18, 20 and 21 under 35 U.S.C. §102(e) over Bronstein et al.:

Claims 1-7, 12-18, 20 and 21 are rejected under 35 U.S.C. §102(e) over Bronstein et al., U.S. Patent No. 6,243,980. The Office Action states that Bronstein et al. teaches a method of detecting the presence of a modifying enzyme in a sample. The reference is said to teach "a first polypeptide that is labeled and comprises a cleavage site for an enzyme, and an antibody (binding partner) that is labeled and specific for a component of the first polypeptide;" "immobilizing the first polypeptide;" "contacting the immobilized polypeptide with the second polypeptide (test sample containing enzyme which is a polypeptide);" "contacting the immobilized polypeptide with said binding partner polypeptide (labeled antibody); and "assaying the modification of at least one of the polypeptides by measuring the association of the binding partner polypeptide to the first polypeptide." The Office Action thus concludes that the reference anticipates the cited claims. Applicants respectfully disagree.

Applicants submit that Bronstein et al. does not teach providing a polypeptide pair comprising a first polypeptide and an second binding partner polypeptide capable of associating, wherein the association of the polypeptides is detectable, and covalent modification of at least one of the polypeptides results in modulation of the association and is required for said association of said first polypeptide and said second binding partner polypeptide, as required by claim 1. Specifically, Applicants submit that Bronstein et al. does not teach the situation where modification of at least one of the polypeptides results in modification of the association, nor does the reference teach that such modification is required for the association. Rather, Bronstein

et al. teaches the use of an anti-fluorescein antibody to detect a fluorescein-labeled HIV protease substrate peptide. The antibody binds the fluorescein regardless of the modification of the fluorescein-labeled peptide substrate. Thus, while the cleavage of the peptide by HIV protease may be covalent modification of at least one of the polypeptides, the cleavage is not required for the association of the first polypeptide and the antibody (second, binding partner polypeptide), as required by claim 1 as amended. Therefore, Bronstein et al. cannot anticipate claim 1 or its dependents 2-7, 12-16 and 20. Applicants also note that the antibody of Bronstein binds to fluorescein, not to the protease substrate peptide. As such, the antibody/fluorescein pair are not a first polypeptide and second binding partner polypeptide, as required by claim 1 as amended.

Finally, while claims 1-7, 12-16 and 20 are distinguished on the grounds discussed above, Applicants note that Bronstein et al. deals only with proteolytic cleavage, and that claim 1 as amended recites that the covalent modification can be one of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications. Proteolytic cleavage is not included as a covalent modification according to claim 1 as amended.

With regard to claim 17, Applicants submit that Bronstein et al. does not teach a polypeptide pair comprising a first polypeptide immobilized to a support, and a second polypeptide bound to the first polypeptide, wherein the binding of the polypeptides is detectable, and covalent modification of at least one of the polypeptides results in modulation of the binding, and is required for said association of said first polypeptide and said binding partner polypeptide. Specifically, Bronstein et al. does not teach that the covalent modification of at least one of the polypeptides is required for the association of the first polypeptide and the binding partner polypeptide. Rather, as discussed above, Bronstein teaches the situation where an antibody against fluorescein tag is bound by anti-fluorescein antibody. The antibody will bind regardless of the cleavage state of the peptide substrate, so such cleavage modification is not required for the association of the first polypeptide and the binding partner polypeptide. As such, Bronstein cannot anticipate claim 17. Further, the types of covalent modification permitted by claim 17 as amended do not include proteolytic cleavage. As such, the claim cannot be anticipated by Bronstein et al.

Claim 18 is novel over Bronstein et al. because the reference does not satisfy the requirement that covalent modification of one or both of the polypeptides by the modifying agent results in modulation of the binding of the polypeptides to each other. The modification (cleavage) of the substrate peptide by the HIV protease taught by Bronstein et al. does not result in the modulation of the binding of the polypeptides to each as required by the claim for at least two reasons. First, as noted above, the polypeptides do not bind to each other at all –rather, the antibody binds to fluorescein tag, not to the peptide substrate itself. Second, the cleavage does not interfere with the antibody's ability to bind the fluorescein tag, in fact, the assay depends upon the continued ability of the antibody to bind the cleaved, labeled peptide fragment. Therefore, the binding of the polypeptides to each other is not modulated by such cleavage. In view of this, Applicants submit that Bronstein et al. does not anticipate the invention of claim 18.

With regard to claim 21, Applicants submit that Bronstein et al. does not teach comparing the association of a first and second polypeptide contacted with a control sample known to contain said modifying enzyme," Rather, the passage cited by the Office Action as providing such a teaching (column 4, line 40) refers to negative controls containing enterokinase and biotin. This is not comparison with a control sample known to contain the modifying enzyme. Therefore, Applicants submit that Bronstein et l. does not anticipate claim 21.

In view of the above, Applicants submit that Bronstein et al. does not anticipate the invention of claims 1-7, 12-18, 20 or 21. Applicants respectfully request the reconsideration and withdrawal of this rejection of these claims over Bronstein et al.

Rejection of claim 17 under 35 U.S.C. §102(e) over Kilburn et al.:

Claim 17 is rejected under 35 U.S.C. §102(e) as being anticipated by Kilburn et al. (U.S. Patent No. 5,962,289). The Office Action states that Kilburn et al. teaches a polypeptide pair, the pair comprising a first polypeptide immobilized on a support, (citing teachings of Kilburn's claims 3, 5-7 and 18-20), and a second polypeptide being bound to the first polypeptide through a cleavage site (citing teachings of Kilburn's claim 2), wherein the polypeptide pair is detectable. The Office Action states that the covalent modification of at least one of the polypeptides results in modulation of the binding, stating "upon cleavage of the site would result in the modulation of

the association of the first and second polypeptides." The Office Action thus concludes that Kilburn et al. anticipates the invention of claim 17. Applicants respectfully disagree.

Applicants submit that Kilburn et al. does not teach a polypeptide pair comprising a first polypeptide immobilized to a support, and a second polypeptide bound to the first polypeptide, as required by claim 17. Specifically, Kilburn et al. does not teach a polypeptide pair at all. Rather, Kilburn et al. teaches a "hybrid protein," comprising a polypeptide fused to an amino acid sequence derived from a polysaccharidase. This is a single polypeptide, not a polypeptide pair. The fact that it has a cleavage site or that it is made up of components of two proteins does not make it a polypeptide pair if it exists as a single polypeptide molecule. Any protein may have a cleavage site for some protease. This does not make it a polypeptide pair. Thus, the single hybrid polypeptide taught by Kilburn et al. is not a polypeptide pair, and the reference cannot anticipate claim 17. Applicants also note that the covalent modification taught by Kilburn et al. is proteolytic cleavage. As discussed above, claim 17 as amended does not include proteolytic cleavage among the covalent modifications recited. Applicants respectfully request reconsideration and withdrawal of this §102 rejection of claim 17 as amended.

Rejection of claims under 35 U.S.C. §103

Rejection of claim 9 under §103(a) over Shone et al, in view of Taremi et al.:

Claim 9 is rejected as obvious over Shone et al. in view of Taremi et al. The Office Action cites Shone et al. as above, but states that the reference "differs from the instantly claimed invention by failing to show the assay method to monitor changes in the molecular mass of the immobilized polypeptide based upon changes in modified polypeptides." The Office Action states that Taremi et al. teaches a method that measures covalent modification of an immobilized polypeptide with an enzyme protease based on changes in molecular mass. The Office Action cites Taremi's teaching "that mass spectrometry provides an accurate measurement of molecular mass and the assay 'is readily adaptable to any enzyme-substrate reaction.'" The Office Action thus concludes that claim 9 is obvious over Shone et al. in view of Taremi et al. Applicants respectfully disagree.

Applicants note that claim 9 is dependent from claim 1. Claim 1 as amended requires the covalent modification to be selected from the group consisting of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications. Both Shone et al. and Taremi et al. teach only proteolytic cleavage. Proteolytic cleavage is the process of cutting a polypeptide into two fragments. Thus, proteolytic cleavage of one of the members of the recited polypeptide pair will result in two fragments of that member of the polypeptide pair. The claims require a covalent modification which is the addition or removal of a chemical group to or from one or both members of a polypeptide pair. The addition or removal of a chemical modification is distinctly different from cleavage via proteolysis. Chemical modifications such as phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization and ADP-ribosylation and their reversal involve the addition or removal of a chemical group to or from a member of the polypeptide pair and do not result in the generation of two fragments of a member of the polypeptide pair. The addition or removal of a chemical modification, particularly phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization and ADP-ribosylation, is not taught or suggested in the Shone et al. or Taremi et al. references, alone or in combination. Because neither reference teaches or suggests the addition or removal of a chemical modification including phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, or ADP-ribosylation, Applicants submit that the combination of the references cannot render obvious the invention of dependent claim 9. Applicants respectfully request the reconsideration and withdrawal of this §103 rejection of claim 9.

Rejection of claim 8 under §103(a) over Shone et al, in view of Little et al.:

Claim 8 is rejected as obvious over Shone et al. in view of Little et al. Shone et al. is cited as above, and the Office Action states that it differs from the claimed invention by failing to show the assay method to monitor changes in the molecular mass of the immobilized polypeptide based upon changes in modified polypeptides. The Office Action states that Little et al. teaches a method that measures the covalent modification of an immobilized polypeptide with an endopeptidase based on changes in molecular mass, the advantage being not having to utilize a radioactive label. Little et al. is said to provide motivation to combine by teaching that mass

spectrometry provides an accurate measurement of molecular mass for relatively short polypeptides, especially immobilized polypeptides that comprise a cleavable linker. The Office Action thus concludes that claim 8 is obvious over Shone et al. in view of Little et al. Applicants respectfully disagree.

Applicants note that claim 8 is dependent from claim 1. Claim 1 as amended requires the covalent modification to be selected from the group consisting of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications. The Shone et al. reference relates only to proteolytic cleavage, and Little et al. is cited for its teachings regarding detection of proteolytic cleavage by mass spectrometry. As discussed above, proteolytic cleavage is the process of cutting a polypeptide into two fragments. Thus, proteolytic cleavage of one of the members of the recited polypeptide pair will result in two fragments of that member of the polypeptide pair. The claims require a covalent modification which is the addition or removal of a chemical group to or from one or both members of a polypeptide pair. The addition or removal of a chemical modification is distinctly different from cleavage via proteolysis. Chemical modifications such as phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization and ADP-ribosylation and their reversal involve the addition or removal of a chemical group to or from a member of the polypeptide pair and do not result in the generation of two fragments of a member of the polypeptide pair. The addition or removal of a chemical modification, particularly phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization and ADP-ribosylation, is not taught or suggested in the Shone et al. or Little et al. references, alone or in combination. Because neither reference teaches or suggests the addition or removal of a chemical modification including phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, or ADP-ribosylation, Applicants submit that the combination of the references cannot render obvious the invention of dependent claim 8. Applicants respectfully request the reconsideration and withdrawal of this §103 rejection of claim 8.

In view of the above, Applicants submit that all issues raised in the Office Action have been addressed. Applicants respectfully request reconsideration of the claims.

Respectfully submitted,

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Date

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Version of Amendments marked to show changes:

1. (Four times amended) A method for detecting, in a sample, the presence of a modifying enzyme which covalently modifies a polypeptide, the method comprising the steps of:

a) providing a polypeptide pair comprising a first polypeptide and a second, binding partner polypeptide capable of associating, wherein the association of the polypeptides is detectable, and covalent modification of at least one of the polypeptides results in modulation of the association and is required for said association of said first polypeptide and said second, binding partner polypeptide, and wherein said covalent modification comprises one of the group consisting of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications;

b) immobilizing the first polypeptide to a physical support;

c) contacting the immobilized polypeptide with the second, binding partner polypeptide;

d) contacting said immobilized polypeptide and said second, binding partner polypeptide with said sample; and

e) [assaying the modification of at least one of the polypeptides by] measuring the association of the second, binding partner polypeptide to the first polypeptide, thereby determining the covalent modification of at least one of said polypeptides, whereby the presence of a modifying enzyme is detected.

17. (Amended) A polypeptide pair comprising a first polypeptide immobilized to a support, and a second binding partner polypeptide bound to the first polypeptide, wherein

the binding of the polypeptides to each other is detectable, and

covalent modification of at least one of the polypeptides results in modulation of the binding, and is required for said association of said first polypeptide and said second binding partner polypeptide, wherein said covalent modification comprises one of the group consisting of phosphorylation, acylation, geranylation, glycosylation, ubiquitination, prenylation, sentrinization, ADP-ribosylation and the reversal of these covalent modifications.

21. (Amended) A method for detecting, in a sample, the presence of a modifying enzyme which covalently modifies a first polypeptide, the method comprising the steps of:

a) providing a first polypeptide immobilized on a support, wherein said first polypeptide comprises a binding site to which a second polypeptide specifically binds, and wherein covalent modification of said first polypeptide detectably changes the association of said first and second polypeptide;

b) providing said second polypeptide and said test sample, and contacting said second polypeptide and said test sample with said first polypeptide immobilized on a support;

c) measuring association of said first polypeptide with said second polypeptide; and

d) comparing said association with the association of a first and second polypeptide contacted with a control sample known to contain said modifying enzyme wherein a change in the association of said first and second polypeptide determined in step (c) relative to said association determined in step (d) provides an indicator of the presence of the enzyme in said test sample.